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LABORATORY EVALUATION OF DIFENACOUM AGAINST THE RICE FIELD RAT, *RATTUS ARGENTIVENTER*

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RINGKASAN

Racun tikus difenacoum telah didapati berkesan terhadap *R. argentiventer*. Takaran tunggal (oral) LD_{50} bagi tikus jantan ialah 0.82 mg/kg dan tikus betina pula ialah 0.68 mg/kg. Kepekatan racun pada 0.001% membunuh tikus jantan adalah tempuh purata (LFP_{50}) 1.17 hari dan tikus betina dalam 1.28 hari. LFP_{50} dan LFP_{99} tergembling (pooled) untuk kedua-dua jantina ialah 1.23 hari dan 3.59 hari. Dalam ujian pemakanan sehari tanpa pilihan didapati bahawa difenacoum pada kepekatan 0.005%–0.03% boleh menyebabkan kematian 70%–100% ke atas kedua-dua jantina tikus-tikus ujian. Tikus jantan mati (purata tempuh sebelum mati) selepas 7.9 hari setelah memakan racun dan tikus betina pula mati selepas 7.6 hari. Ujian-ujian perasa menunjukkan tikus-tikus ujian boleh merasai difenacoum pada kepekatan 0.001 peratus. Berdasarkan kepada ujian-ujian di atas, difenacoum boleh digunakan di sawah pada kepekatan antara 0.005%–0.03 peratus.

INTRODUCTION

Difenacoum, 3–(3–biphenyl–4–yl–1, 2, 3, 4 – tetrahydro–1–naphthyl)–4–hydroxycoumarin, is one of a series of novel 4–hydroxycoumarin derivatives with high anticoagulant activity (HADLER and SHADBOLT, 1975). Laboratory and field studies have indicated that difenacoum is an excellent rodenticide against *Rattus norvegicus* Berkenhout, including warfarin-resistant populations in the United Kingdom (HADLER, REDFERN, and ROWE, 1975; RENNINSON and HADLER, 1976). It has also shown good rodenticidal properties against a wide range of other rodent species (BULL, 1976). This paper described the laboratory evaluation of difenacoum against *Rattus argentiventer* (Robinson & Kloss).

MATERIALS AND METHODS

Technical grade difenacoum with 95% a.i. was used in the tests. All doses are expressed as mg/kg and refer to milligrams of difenacoum per kilogram of body weight. The following tests were conducted against *R. argentiventer*.

i) determination of the single-dose oral LD_{50} (rats used were from an out-bred colony);

ii) feeding tests under 'no-choice' and 'choice' conditions (wild rats caught from the rice fields of Province Wellesley, Penang were used in the no-choice tests and laboratory-bred rats were used in the choice tests).

The test conditions and methods were as given in LAM (1980) and largely followed the WORLD HEALTH ORGANISATION (1982) test procedures.

For the feeding tests, a 'master-mix' containing 0.5% difenacoum was first prepared by mixing the technical grade material with finely ground rice. The toxicant was presented in a bait-base as described by LAM (1979). The dose/mortality and feeding period/mortality data were analysed by probit analysis (FINNEY, 1971), using DAUM's (1970) computer programme.

RESULTS

Single-dose Oral Toxicity

Results of oral intubation with difenacoum are given in *Table 1*. The dose/mortality data were subjected to probit analysis and the results are summarised in *Table 2*. The LD_{50} for males and females, with 95% fiducial limits, were 0.82 mg/kg

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Table 1. Results of oral intubation with difenacoum against laboratory-bred *R. argentiventer*

Sex	Mean body weight (g)	Dose (mg/kg)	Mortality (dead/tested)	Days to death	
				Mean	Range
M	135.3	0	0/10	–	–
M	134.8	0.33	1/20	4.0	–
M	135.0	0.50	4/20	13.8	7–18
M	135.0	0.70	6/20	9.2	7–12
M	135.8	1.00	11/20	7.4	6–10
M	136.4	1.60	20/20	7.5	5–13
M	135.9	2.40	10/10	7.1	4– 9
F	125.5	0	0/10	–	–
F	126.4	0.22	0/20	–	–
F	124.3	0.33	0/20	–	–
F	124.7	0.50	1/20	5.0	–
F	125.8	0.70	11/20	8.4	6–11
F	125.0	1.00	20/20	6.3	4–10
F	125.7	1.60	10/10	6.7	4–10

M – Male
F – Female

Table 2. Probit analysis of data from oral intubation tests with difenacoum against laboratory-bred *R. argentiventer*

Sex	Regression equation[1]	Chi sq.	d.f.	LD_{50} (mg/kg)	95% fiducial limits of LD_{50} (mg/kg)	LD_{95} (mg/kg)	95% fiducial limits of LD_{95} (mg/kg)	LD_{99} (mg/kg)	95% fiducial limits of LD_{99} (mg/kg)
M	Y = 4.82X + 5.42 (0.82)*	4.02	3	0.82	0.71–0.96	1.79	1.40–2.83	2.48	1.81–4.56
F	Y = 13.65X + 7.33 (3.04)*	0.39	3	0.68	0.62–0.74	0.89	0.80–1.14	1.00	0.87–1.39

*Figures in parenthesis denote the standard error of the slope of regression line.
[1]Lines were found to contradict the hypothesis of parallelism (Chi square value = 9.7024; d.f. = 1).

(0.71–0.96 mg/kg) and 0.68 mg/kg (0.62–0.74 mg/kg) respectively. Females appeared to be slightly more susceptible than males although there was no significant difference between the LD_{50} values.

Feeding Tests

(a) No-choice feeding trials

No-choice feeding trials were conducted with difenacoum at concentrations of 0.001%–0.03%. Results of no-choice tests with 0.001% difenacoum are summarised in *Table 3*. The dose (feeding period)/mortality data of 0.001% difenacoum *(Table 3)* were subjected to probit analysis and the results are given in *Table 4*. The median lethal feeding period (LFP_{50}), with 95% fiducial limits, for male and female *R. argentiventer* were 1.17 days (0.60–1.56 days) and 1.28 days (0.84–1.66 days) respectively *(Table 4)*. There was no significant difference in the LFP_{50} between males and females and the data were pooled for probit analysis. The pooled LFP_{50} of 0.001% difenacoum against *R. argentiventer* was 1.23 days (0.93–1.48 days) and the pooled LFP_{99} was 3.59 days (2.61–7.76 days) respectively.

Table 3. Results of no-choice feeding tests with 0.001% difenacoum against wild *R. argentiventer*

Sex	Mean body weight (g)	Feeding period (days)	Mortality (dead/ treated)	Mean bait intake (g)		Lethal dose of poison (mg/kg)		Survived dose of poison (mg/kg)		Days to death	
				Last day of prebait	First day of poison	Mean	Range	Mean	Range	Mean	Range
M	177.6	1	4/10	7.84	7.69	0.54	0.42–0.65	0.36	0.07–0.51	9.3	9–10
F	136.9	1	3/10	6.25	5.26	0.51	0.43–0.65	0.34	0.15–0.59	7.7	5–12
M	206.0	2	8/10	9.57	6.93	0.78	0.55–1.27	0.27	0.21–0.34	9.1	8–12
F	146.1	2	8/10	8.38	6.51	1.04	0.60–1.97	0.41	0.35–0.47	7.1	5–11
M	192.2	3	10/10	6.97	7.01	1.23	0.60–1.93	–	–	7.1	5–11
F	149.0	3	10/10	5.71	5.62	1.29	0.75–2.05	–	–	7.8	5–13

Table 4. Probit analysis of data from no-choice feeding tests with 0.001% difenacoum against wild *R. argentiventer*

Sex	Regression equation	Chi sq.	d.f.	LFP_{50} (days)	95% fiducial limits of LFP_{50} (days)	LFP_{95} (days)	95% fiducial limits of LFP_{95} (days)	LFP_{99} (days)	95% fiducial limits of LFP_{99} (days)
M	Y = 4.63X + 4.68 (1.59)*	0.62	1	1.17	0.60–1.56	2.65	1.90–9.79	3.72	2.41–26.69
F	Y = 5.38X + 4.42 (1.66)*	0.46	1	1.28	0.84–1.66	2.60	1.93–6.66	3.48	2.38–13.57
(M+F)	Y = 4.99X + 4.55 (1.15)*	1.07	1	1.23	0.93–1.48	2.62	2.06–4.47	3.59	2.61– 7.76

(M+F) – pooled data of males and females.
*Figures in parenthesis denote the standard error of the slope of regression line.

Results of no-choice tests with 0.002%, 0.005%, 0.01% and 0.03% difenacoum are summarized in *Table 5*. Difenacoum at 0.005%--0.03% caused 70%–100% mortalities in both sexes in one-day tests. There was considerable variation in the susceptibility to difenacoum among the rats tested. One female survived a dose of 3.35 mg/kg in the one-day test with 0.03% difenacoum, compared with the lowest lethal dose of 0.43 mg/kg of a female in the one-day test with 0.001% difenacoum (*Table 3* and *Table 5*). In the case of males the lowest lethal dose was 0.42 mg/kg and the highest dose survived was 0.72 mg/kg (*Table 3* and *Table 5*).

Mean days to death or average time to die (combined data from no-choice tests) for males was 7.9 days (range 4–15 days) and females 7.6 days (range 4–13 days). No significant difference was detected in the mean days to death between the sexes ($t = 0.06$, $df = 208$, $p > 0.05$). There was no evidence that difenacoum at higher concentrations caused death more rapidly (*Table 5*).

(b) Choice-tests

Choice feeding trials were conducted with 0.001%–0.05% difenacoum and results obtained are given in *Table 6*. Male rats were found to be able to detect difenacoum at 0.001%, the lowest concentration tested. Although significant difference in preference between plain and poison baits was not detected in the females, 7/10 of the females were found to consume more plain baits.

Of the several concentrations tested, 0.01% difenacoum was the most effective, inducing 80% mortality in both sexes. Results indicated that difenacoum at higher concentrations (0.03% and 0.05%) was unpalatable to *R. argentiventer (Table 6)*.

DISCUSSION

Difenacoum was found to be highly toxic (LD_{50} –0.82 mg/kg for males and 0.68 mg/kg for females) against *R. argentiventer*. Among the anticoagulants evaluated against *R. argentiventer* to date (LAM, 1979; 1980;

Table 5. Results of no-choice feeding tests with 0.002%, 0.005%, 0.01% and 0.03% difenacoum against wild *R. argentiventer*

Sex	Mean body weight (g)	Concentration of poison (%)	Feeding period (days)	Mortality (dead/ treated)	Mean bait intake (g)		Lethal dose of poison (mg/kg)		Survived dose of poison (mg/kg)		Days to death	
					Last day of prebait	First day of poison	Mean	Range	Mean	Range	Mean	Range
M	164.8	0.002	1	6/10	8.15	7.11	1.09	0.96– 1.31	0.61	0.52–0.72	7.2	5–11
F	145.8	0.002	1	4/10	6.23	5.33	0.97	0.74– 1.29	0.62	0.46–0.76	6.8	4– 8
M	216.7	0.002	2	8/10	10.57	7.34	1.73	0.78– 3.30	0.24	0.03–0.45	8.0	5–13
F	122.6	0.002	2	10/10	8.38	5.97	2.12	1.00– 3.26	–	–	7.8	5–12
M	192.5	0.002	3	10/10	9.91	8.26	2.87	2.04– 4.39	–	–	8.5	6–12
F	148.3	0.002	3	10/10	7.88	6.91	3.27	2.60– 4.53	–	–	6.4	4–10
M	180.1	0.002	4	10/10	8.73	7.76	3.65	2.43– 4.47	–	–	7.0	5–10
F	138.7	0.002	4	10/10	7.86	6.41	3.75	2.58– 5.43	–	–	6.7	4– 9
M	174.7	0.005	1	7/10	6.75	4.65	1.69	1.03– 2.14	0.64	0.59–0.72	7.0	5–12
F	147.8	0.005	1	7/10	5.31	4.14	1.64	0.83– 2.53	0.99	0.76–1.14	7.4	5–10
M	195.3	0.005	2	10/10	6.58	6.42	3.35	2.32– 4.92	–	–	7.2	4–13
F	138.6	0.005	2	10/10	7.20	6.58	4.76	2.67– 6.38	–	–	7.1	4–11
M	169.9	0.01	1	9/10	7.54	5.54	3.29	2.35– 7.25	0.38	–	7.7	5–10
F	116.5	0.01	1	10/10	5.89	5.56	4.79	2.24– 6.83	–	–	8.3	5–12
M	162.2	0.03	1	10/10	6.61	5.82	11.11	3.65–16.56	–	–	8.5	6–15
F	153.2	0.03	1	9/10	6.92	5.79	12.23	8.11–15.34	3.35	–	7.4	5–11

Table 6. Bait consumption and mortality of laboratory-bred *R. argentiventer* given a choice between plain and difenacoum baits for two days

Sex	Mean body weight (g)	Concentration of poison (%)	Mean daily bait intake (g)		No. of rats preferring plain	t value[1]	Mortality (dead/treated)
			Plain	Poison			
M	183.9	0.001	4.09	2.95	8/10	2.72*	1/10
F	132.2	0.001	3.59	2.36	7/10	1.69ns	3/10
M	223.7	0.002	6.06	2.51	9/10	3.68**	3/10
F	182.3	0.002	5.02	2.83	8/10	2.69*	7/10
M	244.1	0.005	5.57	2.56	9/10	2.82*	7/10
F	170.1	0.005	4.36	1.54	10/10	4.10**	5/10
M	244.7	0.01	5.81	2.34	9/10	3.07*	8/10
F	166.4	0.01	3.58	2.25	7/10	1.49ns	8/10
M	193.2	0.03	6.62	1.26	9/10	4.02**	8/10
F	154.3	0.03	4.29	1.13	9/10	4.83**	7/10
M	184.4	0.05	7.72	0.59	9/10	5.24**	4/10
F	131.4	0.05	6.81	0.71	10/10	8.27**	8/10

[1]* significant at $p<0.05$
** significant at $p<0.01$
ns not significant at $p>0.05$.

1984), difenacoum was found to be more toxic than warfarin (LD_{50} −315 mg/kg) and coumatetralyl (LD_{50} −4.37 mg/kg for males and 2.11 mg/kg for females) but was less toxic than brodifacoum (LD_{50} −0.17 mg/kg). Results also indicated that difenacoum could induce death with a single-feeding, as indicated by the one-day no-choice tests *(Table 5)*. Brodifacoum and coumatetralyl also showed similar rodenticidal action

against the same species (LAM, 1980; 1984). Difenacoum at 0.005% showed a similar level of efficacy against both *R. argentiventer (Table 5)* and *R. norvegicus* in two-day no-choice tests (HADLER *et al.*, 1975).

As observed in warfarin, coumatetralyl and brodifacoum, *R. argentiventer* also showed considerable variation in the susceptibility to difenacoum. Such variable susceptibility could probably give rise to the prospect of difenacoum resistance developing in treated populations after prolonged use. However, a 4-day feeding on a sole diet containing 0.001% difenacoum, based on the pooled LFP_{99}, would be suitable as a screening test for the detection of resistance to difenacoum for *R. argentiventer*.

Palatability tests (choice tests) indicated that rats were sensitive to the presence of difenacoum even at the lowest (0.001%) concentration tested. However, the mortality in the test rats continue to increase up to 80% at the 0.01% level, although at 0.01%, 70%-90% of the rats showed preference for the plain baits *(Table 6)*. At 0.03% and 0.05% levels, highly significant reduction in acceptance was observed (less than 1.5 g of 0.03% was eaten and less than 1.0 g at 0.05%) and as a result there was a decline in the mortality of 10% in the females at 0.03% and 40% in the males at 0.05% when compared with 0.01 percent *(Table 6)*. Similarly, HADLER *et al.* (1975) reported that there was some evidence of unpalatability of 0.005% difenacoum, the lowest level tested against *R. norvegicus*. The above studies indicated that difenacoum could be used at 0.005%-0.01% a.i. against *R. argentiventer* in view of its single-feed action. However, the above concentrations need to be evaluated in the field against wild populations.

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SUMMARY

Difenacoum was found to be a good rodenticide against *R. argentiventer*. The single-dose oral LD_{50} for males and females were 0.82 mg/kg and 0.68 mg/kg respectively. The median lethal feeding period (LFP_{50}) of 0.001% difenacoum against males and females were 1.17 days and 1.28 days respectively. The pooled LFP_{50} of 0.001% difenacoum against both sexes was 1.23 days and the pooled LFP_{99} was estimated to be 3.59 days. Difenacoum was found to have a single-feed action and at 0.005%-0.03% levels caused 70%-100% mortalities in both sexes in one-day no-choice tests. The mean days to death for males and females were 7.9 days and 7.6 days respectively. Palatability tests indicated that *R. argentiventer* was able to detect difenacoum at 0.001 percent. Difenacoum at 0.005%-0.03% could be used in the field for the control of *R. argentiventer*.

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